in 3 parts

P-314

Infectious Agents, Hosts in Constant Flux

Evolutionary perspectives are indispensable for studying the dynamics of infectious diseases and antibiotic resistance

Joshua Lederberg

Late in 1997, a *New Yorker* cartoon depicted Chicken Little in a Hong Kong barnyard crying, "The flu is coming! The flu is coming!" Soon thereafter, the warning proved catastrophic for the chickens in Hong Kong when authorities systematically destroyed them for the sake of halting the spread of a serious avian influenza outbreak—one that, for a time, appeared also to threaten the human population in Hong Kong, mainland China, and beyond.

Health authorities in Hong Kong and their collaborators throughout the world expended extraordinary efforts on the H5N1 influenza episode (*ASM News*, April 1998, p. 188). It is an example of intense international collaboration and local responsiveness amid an enigmatic but potentially devastating outbreak. The aggregate response of health authorities was prompt, vigorous, and well-reasoned—protecting not only the residents of Hong Kong but also everyone on the planet.

We owe those authorities a great debt of gratitude. But that debt cannot be measured solely in terms of their vigilance in Hong Kong. Indeed, that same degree of vigilance needs to be global in scope, and it must encompass the entire range of infectious diseases—a formidable challenge, indeed. Similar outbreaks must be anticipated in the wake of the turbulent evolution of our species' coexistence with would-be parasites.

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Examples Show Infectious Diseases in Constant Flux

Consider deaths caused by infectious disease in the United States during the 20th century. Apart from the overall decline in the incidence of infectious disease mortality and its proportion of total mortality, two observations are striking. One is the extraordinary influenza epidemic of 1918. The prospect of a recurrence remains a major concern to public health authorities.

The other noteworthy observation is the recent reversal in the downward trend of deaths attributable to infectious diseases, perhaps a portent of much worse. About half of those deaths arise because of the current human immunodeficiency virus (HIV) epidemic. A good part of the rest is undoubtedly due to nosocomial infection and drug-resistant disease.

Daily and weekly events reinforce the message embodied in those broader infectious disease trends. For example, articles in a typical issue of the *New England Journal of Medicine* (338:633, 1998, and 338:637, 1998) provide a nearly steady stream of reminders that infectious diseases are in a state of flux, ever promising greater risks to their target hosts.

In one such typical example, Sarah E. Valway and coworkers at the Centers for Disease Control and Prevention in Atlanta, Ga., describe an unusually contagious strain of *Mycobacterium tuberculosis*. Although this *M. tuberculosis* strain distributes rapidly from a few index cases, this miniature epidemic did not cause much morbidity or mortality in humans, purportedly because of early isoniazid prophylaxis among those who were exposed and were at risk for being infected. But this strain grows many times more rapidly in mice than do standard strains of mycobacterium. The ecological circumstance that gave rise to this sort of strain, which has all the earmarks of being a new genetic variant, is difficult to imagine.

In another case described in the same issue of the *New England Journal of Medicine*, Richard Bellamy and others from the Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, United Kingdom, and the Medical Re-

search Council Laboratories, Fajara, the Gambia, report evidence for a polymorphism in the human genome that affects susceptibility to *M. tuberculosis*. Proven examples of polymorphisms affecting susceptibility to infectious agents—unlike incidence of genetically determined metabolic disorders—are rare. Typically, such polymorphisms are evident only when an infectious disease hits a very large part of the population. An example would be the set of hemoglobinopathies that are connected with resistance to malaria. In the more recently identified case affecting susceptibility to *M. tuberculosis*, evidence from differences in strains of mice provided a candidate gene that could then be tested to corroborate the human polymorphism.

Humans, Pathogens Partake in a Continuing Evolutionary Drama

Of course, the interplay of environmental and genetic factors affecting both the host and parasite involved in any infectious disease is complicated. In the course of a specific outbreak, historical contingencies as well as broad overlying biological phenomena further complicate the picture. In more general terms, our relationship to infectious pathogens is part of a continuing evolutionary drama: Here we are, here are the bugs, they're looking for food, we're their meal in one sense or another, how do we compete?

In the battle by attrition, humans have a real problem competing with microorganisms. There are just so many of them, they reproduce so much more quickly, they tolerate vast fluctuations of population size as part of their natural history, and they are not hampered with an emotional apparatus or a need to grieve over any of these matters. Microbes have enormous potentiality in terms of their mechanisms of genetic diversity. Their numbers, their rapid fluctuations, and their amenability to genetic change give them tools for adaptation that far outpace what we can generate on any short-run basis in trying to keep up with them.

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For humans, a small fluctuation of 1% in population size is promptly regarded as a major catastrophe in human affairs. So, why are we still here? One can readily imagine the microbe that could wipe out humanity. Certainly, the 1918 influenza epidemic was a close call. If the mortality rate had been another order of magnitude higher, our species might not have survived.

One has to look at this question in a larger context. Our microbial adversaries share an interest in our survival. With very few exceptions—there are none among the viruses, but a few among the bacteria, such as the clostridial spore-forming toxin producers—human pathogens find themselves at a dead end when they have killed their hosts but still need to relocate to another host to survive. Thus, the really severe host-pathogen interactions presumably have resulted in a wipeout of both species. We are the contingent survivors of such encounters because of a shared interest between host and parasite. It is a very delicate balance, one easily disturbed.

Microorganisms Are Extraordinarily Resourceful

Although bacteria and viruses have somewhat different capacities, collectively their resources are incredible. They all multiply rapidly and nearly incessantly, producing huge population sizes on the order of 10^{12}, 10^{13}, or 10^{14}. Generation times for most microorganisms are measured in minutes.

Moreover, intraclonal methods of variation are legion. DNA replication is often error-prone, and there are often specific circumstances in which the constraints of precise replication are turned off in the presence of DNA damage or other injury. Microbes often live amid chemical or physical mutagens, including UV radiation from sunlight, that may go unimpeded to the very core of their DNA.

For those viruses with RNA genomes, replication is particularly error-prone. Because there are no mechanisms for assuring the fidelity of replication, investigators developed the concept of the quasispecies swarm. For many RNA viruses, retroviruses in particular, the rates of mutation are so high that to a close approximation, every particle is genetically different in at least one nucleotide from every other one. Under such circumstances, the members of a viral quasispecies are rapidly evolving as

swarms of genotypes, with no single genotype being totally representative of the full range of possibilities present at a particular moment.

Of course, natural selection plays a substantial role within a quasispecies because of the differential fitness of one genomic type versus another. What is the role of cooperativity during an infection by the member viruses of a quasispecies—for instance, HIV and other retroviruses? For instance, a single viral particle, which is many generations removed from the original competent infector, may be unable to consummate an infection unless other helper viruses in the same cell complement it.

Most viral and bacterial pathogens are haploid, meaning new genetic factors are expressed without any delay. Although mutations may be slow to accumulate, changes leading to drug resistance are typically manifest almost immediately and will be subject to natural selection very promptly.

Phase Variation Another Means for Abrupt Changes

Phase variation is another genetic feature of pathogens that recently was recognized again as an important set of phenomena. It turns up among very many pathogenic bacterium, as well as among the parasites responsible for malaria, trypanosomes, and *Borrelia*. Although phase variations look at first like mutations, they consist of the microbe activating genetic information that had been silenced during a given time. Its re-expression represents an adaptive change in the presence of a hostile or distinctive environment.

The term phase variation was used first to describe the different flagellar antigens of *Salmonella*, which occur in the specific or group phase, associated with the H1 or H2 loci. The phase change arises when one of these loci is silenced. Specifically, a piece of DNA is inverted, thereby moving the promoter from one locus to another, suddenly transforming the serotype from type 1 to type 2. This phenomenon is completely reversible. Many species of site-specific recombinases are capable of scrambling and rescrambling the bacterial genome in order to silence and unsilence genes that may be then carried in an archival state.

Why do some microbes use this mechanism for keeping genes in a cryptic state when there are other ways of regulating gene expression? One possibility is that phase variation represents a means for controlling antigenic factors. Thus a pathogen avoids telegraphing its host that it is carrying even a relic of an alternative antigenic species, which could provoke host immunity before phase variation begins. Thus, the 100-fold modulation of gene expression that typifies many inducible enzymes might not be good enough for a malaria serotype that represents the parasite's capacity to spring a new antigenic surface on its host. The same may hold for other parasites, including trypanosomes. Conversely, total silencing of the "old" epitopes in the face of an immune response is important for the survival of the new variant.

Because this phenomenon apparently is more pervasive than is widely recognized, a good deal more attention is needed to determine how many bacteria carry genes that are usually expressed as well as others that can be uncovered in the course of their adaptation to new environments. Of course, dozens of genes are expressed only in the internal environment of the host. John Mekalanos of Harvard University and M. J. Mahan of the University of California Santa Barbara recently developed methods to explore tricks akin to phase variation to uncover those genes that are not expressed outside the host the microorganisms may infect.

Not surprisingly, such genes are associated with virulence of the microorganism. But these very subtle controls on gene expression indicate how finely tuned the genetic apparatus of a bacterium may be. Indeed, many bacteria lead double lives—one in the host where they act as pathogens, and another with a totally different set of adaptations required to sustain themselves or proliferate as saprophytes outside the host. Quite different repertoires of genes are involved.

Gene Amplifications, Transfers Are Also Important Mechanisms

Gene amplification is another mechanism that often plays a role in microbial adaptations to their hosts. Gene amplification is more complex than merely turning a gene on and off because segments of DNA are duplicated to reach a higher effective dosage for specific genes whose

products help an organism adapt to a particular environment.

Genetic factors also control mutability rates, perhaps further influencing adaptability. For example, some investigators report that virulent bacteria are more likely to carry mutators, genetic factors that enhance mutability above the norm. We should not be surprised at such instability—it occurs merely by relaxation of the intricately evolved safeguards for replication fidelity and genetic stability. However, mutators are surprisingly prevalent among bacteria, suggesting they may play a general role in helping such organisms face a broad range of environmental challenges. Indeed, external stresses on microorganisms may regulate mutability. Mutation rate is not a constant of physics but a variable of nature. We should not be surprised that bacteria are more mutable when subjected to nutritional stress than when living in a perfectly comfortable environment.

Interclonal variation is an even greater wonder. Recombination mechanisms are highly promiscuous. Conjugation can occur between bacteria of widely varying kinds, most often recognized by plasmid transfer, but every now and then by mobilization of chromosomes, which can occur even across kingdoms.

For instance, a bacterium and a yeast or a bacterium and a plant can exchange genes by conjugation or a process that is analogous. One such process involves the genetic exchanges between legumes and their rhizobium-like parasites. Another involves the crown gall bacterium, which transfers genetic material into the chromosomes of its host plant.

Similar phenomena can be anticipated in eukaryotic infections. There are bacteria that deliver DNA intercellularly to their host animals, and there are a handful of examples of genes floating around in viruses and bacteria that almost certainly were of eukaryotic origin. Lateral transfer across kingdoms is not an unknown phenomenon.

Moreover, plasmid interchange, the movement of tiny bits of DNA from one species to another, is not a laboratory curiosity. Instead, it is a general phenomenon, responsible for the very rapid spread of antibiotic resistance among widely different microbial species—one that heightens our concerns about the wide use of antibiotics as feed supplements in animal husbandry. This practice needs to be reexamined very carefully. The biological mechanisms are certainly there to make it easy not only for single antibiotic resistance traits but whole cassettes of resistance to be moved en bloc from one bacterium to another.

More information about the natural history of these phenomena would help us as we monitor emerging infections. Analytical tools are becoming available to understand the extent to which pathogenic species are involved in these processes. In any case, there is very little that seems to limit the genetic plasticity of microorganisms, including pathogens.

Shared Interests Shape Microbial-Host Interactions

Thus, natural selection in microorganisms operates on almost unlimited genetic variability. When microorganisms interact with specific hosts, their shared interests make it difficult to predict what the balance between host and pathogen will be. The potential outcomes are so divergent that predicting them is exceedingly difficult. Several investigators, including Sir Robert May and Roy Anderson in England, Paul Lewald, and Bruce Levin at Emory University in Atlanta, Ga., are developing a theoretical framework for better understanding these phenomena.

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No matter how well framed, that theory will inevitably prove difficult to corroborate. Any history of interaction between a pathogen and host species remains fragmentary at best, making an analysis of all such interactions a target for skepticism. Nonetheless, such analysis can stimulate thinking about particular pathogen and host relationships.

Such relationships appear to be contingently divergent, subject to radical adjustments. Over the long term, they tend toward coadaptation, with the host acquiring resistance to the damaging effects of the pathogen. Resistance may be acquired through mutations and then favored by natural selection or through cultural changes such as hygienic or behavioral practices. On the other hand, humans sometimes adopt behaviors that are self-destructive rather than protective—

a perverse practice that is difficult to fit into an evolutionary model.

Meanwhile, the virulence of pathogens may be mitigated with time if such changes do not compromise their livelihood. Parasitic microorganisms walk a tightrope, with the bug whose host lives another day itself also surviving, propagating, and spreading for a longer period. For example, the many varieties of rhinovirus, which cause common colds, can be considered an extremely successful set of pathogens with several adaptations that facilitate their spread. However, it would be a cause for concern if the rhinovirus someday mutates into a more virulent form, given its capacity for very rapid spread. So little is known about the genetics of the rhinoviruses that evaluating the likelihood of such a change is next to impossible.

Coadaptation, although difficult to substantiate with a detailed microbial population genetic model, is punctuated by short-term flare-ups. Even in a reasonably stable situation, if a member of that population acquired a mutation to accentuate its growth, it would outgrow its neighbors, cause great harm to its host, and act like a cancer cell does in the disciplined population of the somatic organism cell framework.

In the pursuit of the shared interest in muted lethality and in chronicity, the host plays the more obvious role of self-defense against acute individual lethality. This has immediate consequences for fitness and plays into first-order natural selection.

However, the costs and cumbersomeness of evolutionary change in the multicellular host are overwhelming compared to the facility of that change in the parasite population. Group fitness is an elusive—some think a fallacious—concept, and may be a weaker device for evolution. However, at first blush we should look at every convergence as being lodged primarily in the parasite's self-adjustment for persistence.

This form of adaptability may even include the exploitation of the host's immune system to moderate, but not quench, the growth of the parasite and sustain chronicity and carrier states. These are precarious equilibria and may break down in zoonotic transfers or under stress of coinfection or other environmental stresses. Or, a rogue bug may break the rules and bring the house down.

The model is plainly less applicable to acute, highly transmissible infections lacking carrier states.

With HIV infections, there may be many cycles of coadaptation within the host before some mutation occurs or attrition of the immune system tips the balance and the host becomes severely ill and dies, not of the HIV infection but of other disease. That is what dooms the HIV-infected population.

Part 2 of this article will appear in the February 1999 issue of ASM News.